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BULLETIN No. 12. V. P. P.—54.

U. S. DEPARTMENT OF AGRICULTURE.

DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.

A BACTERIAL DISEASE

OF THE

TOMATO, EGGPLANT, AND IRISH POTATO.

(*Bacillus solanacearum* n. sp.)

BY

ERWIN F. SMITH,
ASSISTANT PATHOLOGIST.

Issued December 19, 1896.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY,
Washington, D. C., August 26, 1896.

SIR: I have the honor to transmit herewith a technical bulletin on a bacterial disease of the tomato, eggplant, and Irish potato, by Dr. Erwin F. Smith. The bulletin is intended mainly to put on record the results of investigations concerning the life history of the organism causing the disease and to set forth certain suggestions in the way of preventive measures that might be adopted. I would respectfully recommend that the bulletin be published as No. 12 of the regular series of this division.

Respectfully,

B. T. GALLOWAY,
Chief of Division.

Dr. CHAS. W. DABNEY, Jr.,
Acting Secretary of Agriculture.

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A BACTERIAL DISEASE OF THE TOMATO, EGGPLANT, AND IRISH POTATO.

(*Bacillus solanacearum* n. sp.)

HISTORY OF THE INVESTIGATIONS.

Attention was first drawn to this disease by statements of Dr. Byron D. Halsted attributing it to the same micro-organism as that causing the bacterial wilt of cucumbers and cantaloupes.[1]

[1] Dr. Halsted has published the following papers and notes on this subject: (1) "Notes upon bacteria of cucurbits" (Proc. Am. Assn. Adv. Sci., Vol. XL, 1891, p. 315); (2) "Bacteria of the melons" (Bot. Gaz., Nov., 1891, p. 303); (3) "An investigation of tomato blight, a blight of potatoes, bacterial melon blight" (N. J. Agr. Exp. Sta. 12th Ann. Rep., and N. J. Agr. Coll. Exp. Sta. 4th Ann. Rep., 1891, pp. 267–276); (4) "The Southern tomato blight" (Miss. Agr. and Mech. Coll. Exp. Sta. Bull. No. 19, 1892); (5) "Southern tomato blight at the North" (Garden and Forest, Aug. 10, 1892, p. 379); (6) "The fungous diseases of the muskmelon" (N. J. Agr. Coll. Exp. Sta. Bot. Dept. Rep., 1893, p. 353; ibid., p. 426). Two distinct diseases appear to be confused in these writings.

It is possible that this disease is the same as that described by Prof. T. J. Burrill in "Preliminary notes upon the rotting of potatoes" (Proc. 11th Ann. Meeting Soc. Prom. Agr. Sci., 1890, pp. 21, 22), and in "An additional note on the rot of potatoes" (ibid., 1891, p. 29). The account, both of the disease and of the organism, however, is too brief and incomplete to warrant any positive conclusion. In 1890 a parasitic bacterium was isolated from rotting potato tubers shipped from the South, and in 1891 the same organism was isolated from the parts above ground. The origin of the material is not given and the micro-organism is not described.

Judging from a paragraph in his Kritische Uebersicht derjenigen Pflanzenkrankheiten, welche angeblich durch Bakterien verursacht werden (pp. 12, 13), relative to the wet rot of potatoes, Dr. Migula has probably also seen this disease in Germany. More recently Wilhelm Biel has described an organism accidentally found in a laboratory at Kiel which in many respects resembles the one here described, but is not identical (Centralbl. Bakt. Allg., Bd. II, p. 137; see also *Ibid.*, p. 572.

A bacterial disease of potatoes and tomatoes has also been reported by Henry Tryon from Queensland, where it is said to be very destructive. See a "New Potato Disease" in Annual Report Queensland Department Agriculture, 1893–94, Brisbane, 1894, pp. 2–4, and part of a paragraph in a paper on "Gumming of Cane," in ibid., 1894–95, p. 14, Brisbane, 1895. In the article on "Gumming of Cane," the potato and tomato organism is called *Bacillus vascularum solani*, but no description is given, and I have not been able to find any, or to decide from the brief account of symptoms whether or not the Australian disease is identical with our own.

During the course of my study of the cucumber disease I made, at different times, more than fifty, perhaps as many as a hundred, inoculations of pure cultures of this organism into the green stems and other organs of tomato and potato plants, all with negative results. Fresh cultures, swarming with motile rods, produced no effect on the potato or tomato, either immediate or remote, while small quantities from the same cultures multiplied enormously when introduced by needle pricks into the green parts of cucumber and muskmelon plants, and invariably produced a wilt of the foliage, usually in from one to two weeks. These experiments were repeated so many times and with such uniform results as to leave no doubt that the tomato and potato are both exempt from attack by the bacillus of the cucumber wilt. The inoculated vines were of various ages, and after inoculation were kept under observation from three to twelve weeks—i. e., until after the tomatoes had matured healthy fruits and the potatoes sound tubers. In some cases a microscopic examination was also made of the stem at the points where the needle entered, but no bacterial lesions were found, and in most cases no bacteria whatever were to be seen. It is impossible, therefore, to come to any other conclusion than that the cucumber and muskmelon disease described by Dr. Halsted is entirely different from that investigated by the writer and shown to be due to *Bacillus tracheiphilus*.

Inasmuch as Dr. Halsted did not experiment with pure cultures and did not obtain very conclusive results from the inoculation of living potato and tomato plants with the Southern potato or tomato rot, it seemed worth while to isolate the organism and determine its life history, the experiments being undertaken at the suggestion of Prof. S. M. Tracy, Director of the Mississippi Agricultural Experiment Station, and carried on partly with a view of determining whether by any possibility the suspected parasite could be the same as that isolated from the cucumber.

In the spring of 1895, on several different occasions, diseased tomato plants were received from F. S. Earle, Ocean Springs, Miss., and in May of that year an extensive series of microscopic examinations, artificial cultures, and plant inoculations was begun in the laboratories and greenhouses of the United States Department of Agriculture in Washington. These were continued for several months with very successful results, an organism wholly unlike that of the cucumber wilt being obtained, and by means of this numerous satisfactory infections of living tomato and potato plants.

On cutting open badly diseased tomato stems (Mississippi specimens) the pith was usually found disorganized into a soft, stinking slime, swarming with bacteria; the woody cylinder was of a brownish hue, and was filled with bacteria; sometimes also the bark was invaded and soft. Agar cultures made from stems in this stage of the disease yielded a variety of organisms. One stained the agar a decided green,

another caused copious evolution of gas both in potato and in peptonized beef-broth agar cultures; some were nearly odorless, while others developed a variety of vile smells. These and subsequent experiments showed that the soft-rotten, bad-smelling stems contained a variety of bacteria.

Tomato stems in an earlier stage of the disease presented quite a different appearance. The foliage had only recently wilted, and the stem was firm and normal in appearance, or nearly so. On section, the pith and bark appeared to be normal, and usually were free from bacteria, but the woody cylinder, or some portion of it, had become brownish, and its vessels, although free from fungi, were plugged with an inconceivable number of bacteria. These oozed out of the vessels, soon after a cross-section was made, in tiny drops of a dirty white or yellowish white color. This ooze was not gummy or sticky, and had no pronounced odor. It was composed entirely of bacteria. The organisms were short rods, often joined in pairs, with a plain constriction between the two. Many were sluggishly motile.

When segments of the stems were placed in 75 per cent alcohol the bacterial slime diffused out of the vessels readily, and this diffusion took place to some extent even in 95 per cent alcohol. The organisms were held in place pretty well by absolute alcohol and by some other quick fixatives, but of course not by chromic acid in water.[1]

As already stated, this organism occurs in the vessels in enormous quantities, and can be traced in woody parts of the affected tomato stems for long distances, even to the top of the vine and out into the petioles, and as the disease progresses it also invades the parenchyma.

Agar cultures from the upper part of stems in this stage of the disease—i. e., prior to the invasion of the parenchyma and the appearance of any stinking smell—gave a rather copious growth of a dirty white or yellowish white organism, not sticky, destitute of vile odor, and consisting of sluggishly motile short rods. In potato broth and beef bouillon there was a slight tendency to pellicle, and a much more copious precipitate than in case of the cucumber wilt bacillus.

Repeated inoculations, at first with bacilli taken directly from the interior of stems in an early stage of the disease and subsequently with pure cultures from tubes of slant agar, led to identical results. There was at first—i. e., for several days—no indication of disease beyond a slight blackening close around the stabs, which were made with a small steel needle, flamed each time before using. Subsequently there was a little sinking in of the tissue, with a slight but characteristic and progressive yellowing of the stem on the pricked side, especially above and below the punctures. Following this, but usually not until after several weeks (the tomato plants were full grown and rather woody), the

[1] *Bacillus tracheiphilus*, the cause of wilt in the cucumber, muskmelon, squash, pumpkin, etc., diffuses out of the vessels readily in aqueous fixatives, and in 50 per cent alcohol, but is held in place satisfactorily by 75 per cent alcohol.

entire foliage of the inoculated shoots wilted and died. In several cases this wilting and death did not occur until a long time after the inoculations. In one large vine (5 feet long), with many extensive branches, several inoculations were made on the terminal parts of a long branch, and in course of some weeks the wilt appeared and a microscopic examination showed the vessels of the upper and middle part of the branch to be full of bacteria. The whole plant, however, did not contract the disease and wilt until at least an additional six weeks had passed, when the bacillus was found plugging the vessels of all parts of the vine, even of branches remote from the one which was inoculated, and death was speedy.

An examination of sections made from the inoculated tomato vines as soon as the wilt appeared gave results identical with those obtained directly from the Mississippi specimens in early stages of the disease—i. e., absence of the bacillus in the parenchyma, slight browning of the vascular ring, and vessels gorged with a bacillus which oozed out on cross-section in the form of tiny, dirty white or faintly yellowish white drops. These drops were not sticky, were destitute of strong odor, diffused out of the vessels readily in 75 per cent alcohol and to some extent also in 95 per cent alcohol (finally all specimens designed for microtome sections were put at once into absolute alcohol), and consisted of short rods, often in pairs and sluggishly motile.

This organism was also inoculated into a number of vigorous young potato shoots. The result was even more striking than in case of the tomato. At first there were no symptoms, but after a few days there was a slight browning, or blackening, and shrinking of the stem in the vicinity of the punctures, and in about two weeks a very decided wilt of the foliage appeared. This was accompanied by a peculiar and very characteristic change in the color of the stems—i. e., from a bright, transparent green to a dull, muddy green, and was followed by the gradual shriveling and death of the stems from the top downward (see Pl. I, fig. 1).

In early stages of this potato disease the bacillus was confined to the vascular system, but the soft parenchyma of the pith and bark was soon invaded and broken down and shriveling followed, the stems being less woody and much less resistant than those of the tomato plants previously mentioned. The vile odor emanating from some, but not all, of the Mississippi specimens was absent.

These potato shoots were about 2 feet high when inoculated, and were punctured well toward the top, mostly on the stem, but in some cases only on single leaflets, the results being the same, except that general infection of the plant did not take place so quickly when the needle pricks were confined to the leaflets.

Some weeks after the stems had wilted and shriveled the earth was knocked out of the pots and the tubers examined. In a number of cases, although not overwatered, the tubers were partly rotten. When

only slightly affected and in condition for study, it was found that the bacillus had entered the tuber through the stem end and had confined its ravages to the vascular ring and its immediate vicinity. The skin of such tubers was unbroken and the outer parts of the bark and nearly the whole of the large central mass of starch-bearing parenchyma were still unmolested. On cutting across such a tuber the vascular ring, or parts of it, was seen to be discolored (brown or black) and tiny drops of bacterial slime oozed out. These were dirty white or faintly yellowish white, did not give off any noticeably bad odor, were not in the least sticky, and consisted entirely of bacilli, some of which were motile. Very often only the stem end of the tuber was affected, and the brownish bacillus-laden vessels, situated on the periphery of the tuber and widely separated from each other by healthy masses of white starch-bearing parenchyma, were easily traced back to one source of infection, namely, the vessels of the small underground stem connecting the tuber with the parent plant, and through these, in unbroken line, to the upper part of the stem, where the bacillus was originally introduced by needle pricks. In one instance, where digging was long delayed, only the empty skins of the tubers remained, the whole interior having rotted away.

In August, 1895, at Charleston, S. C., the writer found what appeared to be the same disease in eggplants. These plants had made much less than the usual amount of growth, and some were dead or dying, while the foliage of others was just beginning to wilt. On making sections of the stem of freshly wilted plants the well-developed vascular cylinder was found to be discolored (brown), and the vessels were gorged with a short, motile bacillus, oozing out in tiny, dirty yellowish white beads, which were neither malodorous nor sticky, and which closely resembled those seen on cross-section of freshly wilted tomato vines. The bark and pith were not yet invaded, and no fungous threads were mingled with the bacilli. Conveniences were not at hand for making pure cultures of the organism, but the bacterial ooze from stems in this stage of the disease was pricked directly into healthy, vigorous tomato shoots on two different occasions in a garden where the tomato disease had not appeared. Altogether fifteen shoots were inoculated. A bacillus multiplied enormously in these shoots, and the course of the disease was identical with that produced in the greenhouse in Washington earlier in the season with infective material taken from tomatoes grown in Mississippi. There was first a slight blackening around the needle pricks and subsequently a shrinking of the stems on the pricked side, especially lengthwise, with change of color from bright green to a yellowish green. Within the stems there was a browning of the vascular system and a plugging of the vessels (even at long distances from the pricked areas) with a short, sluggishly motile bacillus destitute of any strong odor. The wilt of the foliage appeared tardily, owing, perhaps, to the woody character of the stems and to the very frequent rains. No cases appeared in any of the several hundred checks.

These inoculation experiments of 1895 demonstrated clearly enough the existence of a parasitic disease, but owing to an unavoidable interruption in the work it was impossible to decide whether or not the organism was identical with Kramer's potato rot bacillus.[1] The results already obtained were, therefore, presented at the Springfield meeting of the American Association for the Advancement of Science and a summary of conclusions was published in the proceedings of the Society for 1895, page 191.

It was impossible to do anything more with the disease until the spring of 1896, owing to the death of all cultures during a long absence in the summer of 1895. In May of this year the organism was again isolated, this time from the interior of a wilted eggplant received from W. G. Hinson, Charleston, S. C. On this occasion the colonies were obtained from poured gelatin plates.

All the experiments of 1895 have now been repeated many times on tomato and potato, with results which leave no room for doubt either as to the existence of a new bacterial disease or as to the particular organism to which the disease is attributable. The disease has been produced repeatedly by pure cultures derived from single poured plate colonies, and from the interior of the inoculated plants the same organism has been re-isolated and a second series of successful inoculations instituted. Often the swarms of bacilli inside the inoculated stems turned out to be pure cultures of this one organism. This bacillus has been successfully inoculated into a number of other Solanaceous plants and its behavior studied on a variety of culture media. The investigation is by no means completed; but much has been learned concerning the parasite, and it has been thought best to put this on record.

This bacillus appears to be unlike any organism hitherto described, and in view of its predilection for members of various genera of the family Solanaceæ, the writer suggests for it the name *Bacillus solanacearum*, with the following characterization:

DESCRIPTION OF THE ORGANISM.

Morphology.—A medium-sized bacillus, with rounded ends; often in pairs, with a plain constriction; elliptical, but of variable size, depending on age of culture or the length of time the tissues of the plant have been occupied; usually $1\frac{1}{2}$ to 3 times as long as broad. On cover-glass preparations made from peptone beef bouillon cultures 48 hours old and stained with a water solution of methyl violet, many are 1.5 by 0.5μ, but these measurements must not be taken too literally, since the size depends not only on the age of the culture but also on the kind of stain employed, i. e., on whether or not the cell wall stains. Organism motile, often only sluggishly so, especially when taken from the plant,

[1] Kramer, Bakteriologische Untersuchungen über die Nassfäule der Kartoffelknollen (Österr. Landw. Centralbl., I, 1, 1891, p. 11).

but sometimes very actively motile, especially in young cultures. Flagella much longer than the rod; several—exact number and place of attachment not made out clearly, owing to imperfect preparations (Van Ermengem's method), but apparently arising from any part of the rod. An attempt to stain them by Loeffler's method was unsuccessful. No spores observed either in the plant or in culture media, but the search has not been continued long enough to warrant any opinion as to their existence. Zoogloœa are formed almost from the start in fluid culture media. These gather in the upper layers of the fluid in the form of tiny whitish flecks. When examined microscopically these flecks are seen to be made up of groups of motionless rods which hold together—i. e., are not readily separated by tapping the cover glass. These groups contain from a very few to several hundred bacilli. Sometimes these groups have a motion of their own, but whether this movement is due to the action of peripheral members of the zoogloœa or to free-swimming rods which have become stranded or entangled in the fleck has not yet been made out clearly. These zoogloœa frequently form a thin rim on the test tube at the surface of the fluid, and sometimes a thin pseudo-pellicle, but this is often wanting, and in no case have they been found uniting into a compact, tough surface membrane. Ordinarily all that is noticeable is an excess of turbidity in the upper layers of the fluid. This turbidity becomes uniform on gentle shaking of the fluid—i. e., no fragments of pellicle are left floating on the surface of the fluid, but the tiny white zoogloœa, which have gathered into the upper layers of the fluid as if for more oxygen, are then uniformly distributed through the fluid.

Symptoms produced in the plant.—The first indication of this disease, or at least the first one to attract the farmer's attention, is the sudden wilting of the foliage. This may occur first on a single shoot, but finally it affects the whole plant. Subsequently, and especially if the plant is young or not very woody, the stem shrivels, first changing to a yellowish green or to a muddy green, and finally to brown or black. The vascular bundles become brown long before the shriveling takes place, and in the potato often show through the outer green parts of the stem as long, dark streaks (Pl. I, fig. 1), or the bacteria run out on the petioles, after the manner of pear blight, forming narrow, black lines. The vessels of such bundles are filled with the bacilli, which ooze out when the stem is cut across. The foliage may wilt with or without a preliminary yellowing. If the bacteria are abundant in the vessels of the stem the wilt is often very sudden and the foliage has no time to become yellow. The progress of the disease seems to be more rapid in young than in old plants and in hot than in cold weather.

In the case of the potato the tubers are also finally attacked and destroyed, the organism reaching them by way of the vascular bundles of the stem. A brown or black rot ensues, beginning in the stem end of the tuber in the vascular ring and extending in all directions therefrom. All stages of this rot of the tubers (both in 1895 and in 1896) were

obtained repeatedly from pure cultures of the bacillus pricked into the stem several feet above ground. In some cases the whole interior of the tuber was soft rotten; in others only the vascular ring was badly invaded; in others only a small part of the vascular ring in the vicinity of the stem end of the tuber was attacked; in others the organisms had barely gained access to the tubers (vascular ring) and no lesions were yet visible to the naked eye; and finally, in still other cases the bacilli had not yet reached the tuber, but were moving toward it, the vessels of the upper part (upper one-half, two-thirds, etc.) of the underground stem, which bore the tuber at its extremity, being fully occupied by them, although in most cases the stem retained its shape and firmness and was sound externally. Such underground stems were easily distinguished from healthy ones by their dark color, due to the brown staining of the vascular bundles. Dusky brown patches, due to internal staining, often appeared on the surface of the tuber in advance of the external appearance of the soft rot. When digging was purposely delayed for some weeks after the decay of the stems, the tubers were then very badly rotted or had entirely disappeared.

None of the many checks contracted the disease, not even when grown in the same pot with the inoculated plant, and nothing could be plainer than the manner of infection of the tuber—i. e., by way of the vascular bundles of the stem. In the greenhouse, rot of the tubers was well under way in three to six weeks from the time the stems were inoculated well above ground. In the field, under very favorable conditions, it is quite likely that the rot might appear sooner, or, on the other hand, under unfavorable conditions might be delayed. The rapidity of the development of the disease would, of course, be proportional to the number of separate infections.

Probably the simplest way of distinguishing this disease is by the wilt of the foliage associated with a distinct browning of the vascular system, and with the presence in the vessels of myriads of bacilli. In early stages of the disease these are likely to be all of one kind, but later on many others may gain access to the plant and help to complete its destruction. When the disease is well advanced, as in case of the stem shown in Pl. I, fig. 1, it may also be distinguished by the alkaline reaction of the juice when squeezed out upon litmus paper, the juice of healthy stems giving an acid reaction.

Anatomical changes in the host plants.—These have not been worked out fully. The starch grains seem to be uninjured by this organism. The lignified tissues also resist. The organism attacks and destroys the parenchyma of the pith and bark, and also destroys the protoplasm, converting nearly the whole interior of soft stems, like those of the potato or young tomato, into a watery mass of broken-down cells mingled with bacteria. In old and well-lignified stems, like those of well-grown tomatoes and eggplants, the outline of the stem is better preserved and the lesions are less extensive. In the tubers of the

potato well-defined cavities arise in the vicinity of the vascular ring. These have brown or black walls and are filled with loose starch grains, remnants of cells, and myriads of bacteria. On the borders of these cavities the cells and cell walls of the starch-bearing parenchyma may be found in all stages of solution and decay.

Bouillon and peptone cultures.—This organism grows well, at room temperatures of 20° to 30° C., in beef broth peptonized (Witte's peptonum siccum). It seemed to make little difference whether the bouillon was left acid or rendered slightly alkaline with carbonate of soda. The gathering of the zoogloœa in the upper layers of the fluid is very distinct, especially if the tubes are left undisturbed in an upright position for some days. On shaking the turbidity becomes uniform. The organism produces a copious, dirty white precipitate (much more precipitate than *B. tracheiphilus*).

The behavior in alkaline peptone water (Savory & Moore's brown peptone) is not unlike that in beef bouillon. The organism retains its vitality in these fluids many days, and they are quickly changed from acid or neutral to alkaline. The fluid then gives a strong blue reaction with neutral litmus paper. Nessler's fluid gives an immediate, copious, orange-yellow precipitate. Old peptonized beef broth cultures became brown, those made alkaline by sodium carbonate taking a deeper tint than those left acid. At the end of two to three months the color was very decided. Old peptone water cultures did not brown. The latter culture medium was free from sugar, i. e., yielded no gas with *Bacillus cloacae;* the former contained some muscle sugar, i. e., yielded more or less gas with *B. cloacae.*

Milk.—This proved to be a very suitable culture medium. There is no immediate change, but after some weeks (room temperature 25° to 30° C.) the milk becomes saponified—i. e., is changed from a white, opaque fluid to a pale yellowish, translucent fluid. The casein is not precipitated. No acid is formed in the milk. Such milk becomes strongly alkaline, even when rather acid on the start. The casein remains unchanged, and 1 to 2 c. c. of a one-half per cent solution of hydrochloric acid precipitated it in the form of a bulky white curd, filling the whole fluid. This clearing of the milk is undoubtedly due to a combination of the fat globules of the milk with the alkali developed by the organism during its growth. The same phenomenon may be obtained in a few minutes by adding to a test tube of pure milk fifteen to twenty drops of strong ammonia and shaking. This saponification also takes place in the thermostat at 37° C., but not sooner than in open air at summer temperatures—i. e., only after about three weeks.

Litmus milk.—Two kinds of litmus were used for coloring the milk, namely, (1) a violet fluid, of good keeping quality and unknown composition, made by Sharp & Dohme, Baltimore, Md., and recommended by Dr. William Welch, of Johns Hopkins University; and (2) a freshly prepared, saturated, dark blue aqueous solution of Trommsdorf's c. p.

litmus, which comes in small, irregularly cubical or oblong sticks. Twenty and forty-drop portions of the former and twenty-drop portions of the latter were added to 7 c. c. portions of fresh milk in test tubes. The color of the milk after sterilizing was different shades of lavender, depending on the amount of litmus added. The addition of forty drops of the saturated solution of Trommsdorf's litmus to 7 c. c. of the milk caused, on steaming, a precipitation of the casein, but the lesser quantities of litmus produced no such result.

The inoculated tubes of litmus milk developed no acid—i. e., showed no trace of reddening. After two or three days the litmus became perceptibly bluer than in the control tubes, and this blueing increased from day to day, indicating a progressing alkalinity. This change took place at room temperatures of 20° to 30° C., and also in the thermostat at 37° C. The casein was not precipitated.

Gelatin.—In plate cultures of nutrient gelatin the buried colonies are circular in outline (globose), yellowish or brownish, granular (under Zeiss 16 mm. objective and 12 compensating ocular), and with well-defined margins. No oblong or spindle-shaped colonies could be found. The circular outline and regularity and distinctness of the margin of the colony were especially noteworthy. Whether these features will be found constant with all gelatins is a question yet to be determined. Occasionally, after a few days, a narrow, clear zone appeared around the margin of many of these colonies as if liquefaction had set in. This, however, did not progress, or increased but very slowly, and was clearly visible only under the compound microscope. The buried colonies remained small, as if requiring more oxygen than they were able to get. The surface colonies were circular, thin, thin-edged, smooth, white, and wet-shining. They did not spread over the plate rapidly or cause any liquefaction (15 per cent gelatin, temperatures 20° to 27° C.).

For the sake of comparison, tubes of standard alkaline peptonized beef-broth gelatin were obtained from several different laboratories in Washington and used for streak and stab cultures. In streak cultures the bacillus made a variable amount of growth, depending, apparently, on slight differences in the composition of the nutrient gelatin. This growth was pure white, smooth, wet-shining, and caused no decided or appreciable liquefaction of the gelatin, not even after a month (temperature 20° to 27° C.). Numerous short, finger-like projections were sent down from the under surface of the streak into the gelatin. In stab cultures the bacillus grew best in the upper part of the stab, making a thin, whitish growth. The "nail head" did not spread widely (only a few millimeters). It was pure white, thin edged, smooth, wet-shining, and did not cause any liquefaction of the gelatin for a long time, but appeared to have settled or grown (?) into the gelatin a trifle after five or six weeks (temperature 20° to 27° C.). No gas was developed in the gelatin. The bacillus does not liquefy gelatin, or at least not during the first few weeks of its growth.

The organism grew best in a gelatin of the following composition: Lean minced beef, 500 c. c.; distilled water, 1,000 c. c.; mixed and set twenty-four hours in a cool place; filtered and added 10 grams of Witte's peptonum siccum and 150 grams of L. and F. gelatin. This gelatin was clarified with egg and rendered alkaline with sodium hydrate, titrating with phenolphthalein. The degree of alkalinity was between 12 and 14 of Mr. Fuller's scale.[1]

Agar.—In poured plates of nutrient agar the buried colonies differ considerably from those in gelatin. Instead of being circular, with a very smooth margin, they were irregularly round or even oblong, with a decidedly irregular granular margin. These colonies were brown or yellowish brown under 16 mm. objective and 12 ocular. After some weeks the whole body of the agar became decidedly brown. No spindle-shaped colonies were to be seen. The surface colonies grew rather slowly. They were dirty white, smooth, wet-shining, and did not spread widely over the agar.

Nutrient agar from several different laboratories was also tested with streak and stab cultures. The growth of the streak cultures was variable, but generally abundant, being poorest in some rather alkaline 6 per cent glycerin agars obtained from a neighboring laboratory. The streak was usually well developed in twenty-four to forty-eight hours (temperature 28° to 31° C.). It was smooth, wet-shining, and at first white or dirty white, then usually yellowish to brownish white, and finally brown. In most cases the nutrient agar under the streak was stained brown (pale brown in some tubes and deep brown in others), the depth of color depending apparently on the composition of the agar—i. e., probably either on the amount of muscle sugar in the beef broth used in its preparation or else on some unknown constituent of the sea weed itself. There were no outgrowths from the lower surface of the streak into the body of the agar. In stab cultures the organism grew decidedly better in the upper part of the stab, gradually fading out below and making only a very feeble growth in the depths. The margins of the stab, especially in the upper layers of the agar, pushed outward in the form of scallops or festoons, which sent out into the agar numerous short, granular projections. The surface or "nail head" growth in the stab cultures was thin, smooth, wet-shining, and at first white, then dirty white, then yellowish brown, then brownish. In some tubes it spread wider than in others, depending apparently on the amount of moisture in the surface layers of the agar. No gas was formed. Most of the many agars tried became brown after some days or weeks, especially in the upper part, where the stab grew best. On one agar the bacterial layer remained white (finally dirty white) and the substratum did not become brown until after many weeks, and then only slightly, although the organism made a good growth in it. On adding dextrose to this agar the browning took place sooner.

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[1] Fuller, "On the proper reaction of nutrient media for bacterial cultivation" (Jour. Am. Pub. Health Assn., Oct., 1895, p. 381).

On testing with *Bacillus cloacae*, the beef bouillon from which this agar was made was found to be entirely free from muscle sugar. No trace of gas appeared in the fermentation tube, while a loop of fluid taken therefrom on the fourth day and introduced into a fermentation tube containing fruit sugar peptone water caused a copious evolution of gas on the second and third day. The behavior of this agar emphasizes once more the necessity of knowing in each case whether the beef bouillon contains muscle sugar. If only this one agar had been tried, I should have written unhesitatingly, "Growth white, not imparting any color to the substratum," and the chances are that the very next person would have obtained contradictory results.

The germ grew most readily in an agar of the following composition: Liebig's extract of meat, 2½ grams; distilled water, 1,000 c. c.; Witte's peptonum siccum, 10 grams; agar, 10 grams. The agar was cooked soft over a hot flame in a very small quantity of water, then added to the rest of the water and steamed for some time. The steamed fluid was clarified with white of egg and made feebly alkaline with sodium carbonate. Its alkalinity was probably about 10 to 12 of Mr. Fuller's scale. In the same agar rendered more strongly alkaline by sodium carbonate the growth was less abundant.

Potato cultures.—Large test tubes, containing steamed, sterile potato cylinders, about 2 cm. in diameter and 6 cm. long, were used for these experiments. The upper surface of the cylinder was slanted to receive the germs and the lower end rested in several centimeters of distilled water.

The behavior on potato is very characteristic. In twenty-four to forty-eight hours (temperature 27° to 32° C.) the fluid became turbid and the projecting part of the cylinder was covered with a copious, wet-shining growth. At first this growth was white or dirty white, but after some days (three to ten) it became brown, and finally, in places, nearly or quite black (smoke brown is perhaps the proper term). The growth on potato was not wrinkled. The substratum and the fluid in the bottom of the tube also became brown. The rapidity and the degree of pigmentation seem to depend on the slightly varying composition of the potato, apparently on the amount of glucose present. No gas was formed in any of the many potato cultures. No acid was detected in any stage of the growth of the cultures, not even when tested at the end of the first twenty-four hours. The potato cultures, which were slightly acid on the start (normal acidity of the tuber), soon became strongly alkaline to litmus paper. With Nessler's solution the alkaline potato cultures gave an immediate, copious, orange-yellow reaction, indicating ammonia. These cultures developed a peculiar odor, often noticed in rotting potatoes, but not specially disagreeable. This odor was likened by one person to the smell of sour bran. Its chemical nature has not been determined. The cylinders did not fall into pieces, but retained their shape for several weeks. They

were stained throughout, but not uniformly (deepest at surface), and were only partially softened.

Fermentation tubes.—The behavior of this organism, when grown in fermentation tubes in nitrogenous media, to which the various sugars have been added, is specially interesting. A 2 per cent alkaline peptone water was prepared in large quantity from distilled water and Witte's peptonum siccum. This fluid was then divided into a number of equal portions, a different sugar added to each one (1 gram per 100 c. c.), and the clear fluids pipetted into clean fermentation tubes and sterilized in the ordinary way (three steamings of twenty minutes each on three successive days). In this way the following c. p. sugars and allied substances were tested for gas production and other phenomena: Grape sugar, fruit sugar (Schering's diabetine), cane sugar, milk sugar, maltose, and dextrin. No gas and no acid were formed from any of these carbohydrates, and there was no clouding in the closed end of any of the tubes, not even after several weeks. An impure galactose (Merck's "Galactose pur") also gave the same results. The bacillus made a good growth in the open end and outer two-thirds of the U of each tube, and after a few days all the fluids were decidedly more alkaline to neutral litmus paper than they were on the start, as determined by comparison with check tubes. After some weeks a decided brown tint developed in the fluids containing the grape, fruit, and cane sugars. This was especially noticeable in the open end and in the U. The fluids containing the milk sugar, maltose, and dextrin retained their original color for a long time but, finally, they also became brown. The bouillon containing the impure galactose also browned quickly, but inasmuch as this sugar polarized too low (+ 70), the speedy browning is probably attributable to the presence of a small amount of dextrose. Tubes of the glucose and saccharose bouillon, in which the organism had been growing seven weeks, and which were strongly alkaline, yielded no gas bubbles on acidifying with H_2SO_4, and on distilling no acid other than a slight amount of CO_2 could be detected in the steam.

These fermentation tubes were inoculated from fluid cultures (the second subculture from a poured plate colony), and after the above results were obtained (three weeks after installation of the cultures) the continued virulence of each culture was determined by inoculating potato shoots with bouillon cultures made therefrom, the disease resulting in each case in a few days.

Gas production.—No gas appeared in any of the many cultures. The organism is not a gas producer.

Relation to oxygen.—This bacillus appears to be strictly aerobic. If ever facultative anaerobic it is not so with any of the carbohydrates yet tested.

Acids.—No acid reaction could be detected in any stage of any of the cultures. Potato cultures only twenty-four hours old and which were

acid on the start (normal acidity of the tuber) gave a decided alkaline reaction to litmus paper. If any acid whatever is formed it is masked by the presence of alkali and is not butyric acid.

Alkalies.—This organism is a very vigorous alkali producer. On warming the cultures over a gas flame or on placing the blued strips of litmus paper on a warm glass plate the alkaline reaction quickly disappears. On adding a few drops of Nessler's reagent, as already stated, a copious orange-yellow precipitate is at once developed. This would indicate that at least a part of the alkali is due to ammonia. Probably amine bases are also present.

Temperature relations.—The thermal death point (ten minutes' exposure) has not been determined exactly, but is probably about 52° C. It certainly is above 51° C. and below 53° C.

This conclusion is the result of 14 sets of experiments using an Ostwald-Pfeffer thermostat with a Roux metal bar regulator. Working under favorable conditions, i. e., with a steady gas pressure in the city mains, it was found possible with this apparatus to keep the temperature practically constant, the range during short periods (1 hour) not amounting to more than one-twentieth of 1 degree. The temperatures were taken with a Max Kaehler and Martini thermometer, recently compared for this purpose with the standard hydrogen thermometer of the International Bureau of Weights and Measures, Washington, D. C. All exposures were made in 10 c. c. portions of peptonized beef bouillon in thin-walled test tubes 6 by eleven-sixteenth inches. The tubes were inoculated with large loops from recently clouded bouillion cultures and after about one-half hour a portion of each set was exposed to the heat by plunging the tubes into the water nearly to their top. In most cases the tubes were cooled under the tap five minutes after removal from the bath.

The bacillus grows well in the thermostat at 37° C.—possibly a trifle better than outside at summer temperatures ranging from 25° to 32° C. Under either condition it grows rapidly. It still grew readily from bouillon cultures after several weeks' exposure to 37° C. (three weeks' exposure in one case, seven weeks' exposure in another).

The minimum temperature at which growth will take place has not been determined exactly, but seems to be in the vicinity of 13° C. The organism is not destroyed by cold, at least not very readily. Cultures were easily obtained from a bouillon culture after exposing twenty minutes to −77° C. (−106° F.) in a mixture of frozen carbon dioxide and ether. Glass tubes cracked from the intense cold, and the exposures were finally made in long test tubes of block tin. The bouillon cultures were pipetted in and the tubes placed upright in a mixture of salt and pounded ice until the temperature reached about −10° C. The upper 2 to 3 inches of these tubes projected out of the freezing mixture. The fluid did not reach up to this part of the tubes, but the inner walls were necessarily wetted in transferring the bouillon. This part of the tubes was now heated in a Bunsen flame sizzing hot down

to the level of the freezing mixture to insure the death of all the germs which adhered to the upper part of the tubes. The latter were then buried nearly to their tops in the mixture of frozen CO_2 and ether, both substances being renewed as fast as they boiled away.

Behavior toward stains.—This bacillus takes the various basic anilin stains readily.

Pigments.—A brown pigment is formed in course of a few days in the host plants (potato, tomato, etc.), and in culture media containing grape, fruit, or cane sugar (nutrient agar, steamed potato, fermentation tubes). This pigment is soluble in water and glycerin. It is insoluble in ethyl alcohol, ether, chloroform, xylol, and carbon bisulphide. It is slightly soluble in methyl alcohol on long standing. It is not destroyed after several days' exposure to the following acids and alkalies: Sulphuric acid (10 per cent), nitric acid (10 per cent), hydrochloric acid (5 per cent), oxalic acid (10 per cent), acetic acid (20 per cent), sodium hydrate (1 per cent), sodium hydrate (5 per cent), strong ammonia. This pigment seems to result either from the action on the sugars of the alkali produced by the growth of the germs[1] or else by the action of some other substance, which becomes effective only after the medium has become alkaline. The browning can not take place without the presence of sugar or in the absence of alkali. The action of light is not necessary for the production of this color. On adding strong ammonia to a tube of sterile dextrose peptone water there was an immediate slight browning of the fluid, and this color deepened and became very noticeable after some weeks, whereas sucrose peptone water treated in the same way remained perfectly clear. This would suggest that under the influence of this organism the various sugars are first converted into grape sugar and then some part of the latter into this pigment under the chemical action of the alkali.

HOST PLANTS.

This disease has been observed in the field in tomato (*Lycopersicum esculentum*), potato (*Solanum tuberosum*), and eggplant (*S. melongena*). In the greenhouse, under strict control conditions, and experimenting with pure cultures, it has been produced repeatedly in the tomato and potato, and also a number of times in several other members of the

[1] A browning of the culture media and of the fungus has also been obtained by the writer with the watermelon wilt fungus (*Fusarium niveum*). All that is required to bring about this change is the addition of sugar to an alkaline culture medium. The browning of the vascular system in cotton, watermelon, cowpea, cabbage, and other plants attacked from within by parasitic Fusaria, would seem to be, therefore, in some way dependent on the growth of the fungus in an alkaline medium (sap of the vessels) to which sugar has access (sugar from the cell sap). It is not unlikely that the pigmentation of many ascomycetous fungi is accidental, depending on chemical changes produced by the fungi in various substrata and on the subsequent absorption of the pigments. Results obtained with the above-named fungus seem to point unmistakably to this conclusion.

family Solanaceæ. The cultures used for inoculations in 1895 were obtained from the tomato. Those used for inoculations in 1896 were derived from an eggplant. The Jamestown weed (*Datura stramonium*) takes the disease from needle pricks quite readily. Good cases were obtained in 1895 and again this year. The bacilli multiply enormously inside of the plant and pass down the stem long distances, causing the foliage to wilt and the stem to shrivel. In some cases the germs were pricked into the leaves and in others into the stems. The black nightshade (*Solanum nigrum*) also contracted the disease readily from needle pricks, whether into the blade of the leaf or into the stem. The foliage wilted and the stems shriveled. These results were obtained in 1895 and again this year. In one wilted plant examined two weeks after inoculation, which was by means of a few delicate needle pricks in the upper part of the stem, the entire vascular system for a distance of more than 8 inches down the stem was plugged full of the bacilli. On this plant the first wilted leaf appeared four days after the pricks, and the wilt involved the whole top (twelve leaves) eight days later.

To the list of possible host plants must be added the species of two additional genera, viz, Physalis and Petunia. The species experimented on in 1896 were *Physalis crassifolia*, *P. philadelphica*, and a vigorous white-flowered petunia, a hybrid probably of *Petunia nyctaginiflora* and *P. violaceae*. Several experiments were made and in each case the foliage wilted and the stem shriveled. The petunia, however, resisted much better than the species of Physalis, and was not wholly destroyed, only the parts beyond the pricks in the stems—i. e., the tenderer portions (terminal 6 inches)—shriveled and died, and even these parts contracted the disease slowly.

The following species resisted: *Nicotiana tabacum* (1895 and 1896), *Capsicum annuum* (1895), *Solanum muricatum* (1895), and *S. carolinense* (1895 and 1896). The last three species should be tested further. Attempts to induce the disease in *Pyrus communis*, *Pelargonium zonale*, and *Cucumis sativus* failed. In the first series of experiments on the cucumber four plants were inoculated. Two of the cucumber plants received numerous punctures on the basal part of the blade of a full-grown leaf, and two received numerous deep stabs into one node and two internodes toward the base of the stem, using a virulent culture, which produced wilt and shriveling of a potato stem in six days, and proved equally virulent to *Physalis crassifolia*, *Datura stramonium*, and *Solanum nigrum*. The plants were under observation six weeks, during which time they remained healthy. This experiment was repeated with similar results. The plants resisted perfectly or showed only local symptoms (enlargement and cracking open of pricked internode), although check plants (tomato) readily contracted the disease.

Frequently, however, but not always, the organism seems to multiply when inoculated into cucumber fruits, especially if nearly full grown, and kept under a bell jar in very moist air. If the inoculation takes,

the whole interior of the fruit becomes a soft, wet mass of bacterial slime and disorganized tissues, held in place by the tough, unbroken skin of the fruit, which presents a water-soaked appearance, as described by Dr. Halsted. The manner of infection was to sterilize a square centimeter of the smooth surface of the fruit with a red-hot spatula, plunge a red-hot needle into the center of this spot, and then insert a little of a pure culture of the organism on the end of a platinum wire. Several things have, however, thus far conspired to render these experiments of uncertain value. Owing to the excessive moisture of the air under the bell jar and to the fact that the whole exterior of the fruit was not sterilized, fungi developed here and there on the surface of the fruits and appeared sooner or later in the mouth of the wounds. Some of the inoculated fruits did not rot at all, although subject to the same conditions. No poured plates were made to determine whether the bacteria present in great numbers in the soft interior of the rotted fruits were pure cultures of the germ which was inserted. Until these objections have been removed by further experiments the exact relations of this bacillus to the water-core rot of cucumber fruits must remain undetermined.

Dr. Halsted described his disease as rotting off the stems of melons and squashes at the surface of the earth, and it is not unlikely that this organism may have to do with that trouble, finding its way into the base of the stem through insect borings and completing the destruction begun by various larvæ. This, however, is mere conjecture.

GEOGRAPHICAL DISTRIBUTION.

As yet little is known respecting the distribution of this disease. It occurs in southern Mississippi and Alabama, on the alluvial coastal plain near Charleston, S. C., and near Washington, D. C. No other localities are definitely determined, but the disease is to be looked for in many other parts of the United States. In the potato it almost certainly occurs as far north as New York (Oswego County). Probably it exists in many places and has been confounded with other tomato and potato diseases.

LOSSES.

In southern Mississippi the losses on the tomato crop have amounted to thousands of dollars, and have been so great in many places in recent years as to discourage the cultivation of this fruit for market purposes, entire fields being destroyed year after year. The same conditions exist at Charleston, S. C. The disease is common in that region in tomatoes and eggplants. In 1895 the writer saw whole fields of tomatoes destroyed by it, and earlier in the season it had greatly injured the potato crop grown for the Northern markets. One grower estimated his loss on 5 acres of potatoes at 65 barrels. The disease first appeared as a sudden wilt of the tops of the vines and the

rot of the tubers followed. Many of the tubers that appeared sound on digging rotted on the way to market. The disease is not new to that part of the United States, and it has become a common remark among the more observant potato growers that if the tubers are dug soon after the wilt of the foliage appears they will remain sound—an observation that fits in well with the results of the inoculations already detailed, and leads to the conclusion that the Charleston potato disease can be nothing else than the one here described.

If the potato rot of the northern United States turns out to be the same disease, as seems not unlikely, then it is not only widespread, but the losses are very considerable every year and in certain seasons foot up into hundreds of thousands of dollars. The losses from potato rot in the principal potato-growing States of the United States in 1895, estimated on the basis of reports furnished to this Department, ranged from 5 to 60 per cent of the whole crop.[1] What this really means becomes clear only when we consider the enormous bulk of the annual potato crop of the United States. That of the year 1879 (census of 1880) amounted in round numbers to 170,000,000 bushels, and that of the year 1889 (census of 1890) amounted to 217,500,000 bushels. At a low estimate the loss in 1885 exceeded 50,000,000 bushels, the percentage of loss being greatest in New England, New York, Michigan, Wisconsin, and other great potato-growing regions.

Of this much at least we may be certain, viz, that only a small part of the potato rot of the United States is due either directly or indirectly to the potato mildew (*Phytophthora infestans*).

NATURAL METHODS OF INFECTION.

In the greenhouse, under strict control conditions, the writer has been very successful in transmitting the disease by means of the Colorado potato beetle (*Doryphora decemlineata*). The first experiment was begun July 23, 1896. A handful of the beetles was placed under a bell jar on potato tops taken from plants inoculated July 16. These tops had wilted and were becoming brown. The beetles fed upon them as readily to all appearances as upon healthy shoots. They were then placed for some hours under a large bell jar on a well-grown, healthy plant. This plant was eaten in many places, but was not seriously injured. The beetles were then removed and the plant was placed under normal conditions to await developments. On the eighth day there were slight indications of wilt on a dozen different leaves scattered over the plant, indicating as many separate infections. The weather was warm and after a day or two these symptoms progressed rapidly. The wilting leaves shriveled and in a few days long brown streaks appeared inside of the stems, beginning

[1] See map showing distribution and severity of potato rot in the United States in 1885 (Ann. Rep. U. S. Dept. Agr., 1888).

usually at the base of the shriveled leaves. In ten days from the appearance of the first symptoms the whole vine was involved, all the leaves wilted and shriveled, and the stems became muddy green, blackened in stripes internally, and finally shriveled. Cross-sections of the stem showed the vessels to be gorged with the bacillus. On August 24 the earth was knocked out of the pot and the tubers examined. These were found in all stages of rot.

Three other large, well-grown potato plants were subsequently inoculated in the same way and with the same result (Pl. II). In each case the disease began simultaneously in many different parts of the vine seven to nine days after the beetles were removed, and the tubers were rotted in whole or part three weeks after the appearance of the first symptoms on the foliage. The check plants remained healthy.

These experiments with the Colorado potato beetle seem to fully warrant the conclusion that insect enemies are largely responsible for the spread of this disease. The direct injury resulting from their bites and punctures is not the only injury nor the worst one. Given one diseased vine in a field and plenty of insects to feed upon it, and the transmission of this disease to all parts of the field, and thence to the whole neighborhood, is only a question of a few weeks.

Just what insects are most instrumental in disseminating this parasite in any particular locality can be determined only after a prolonged and careful study of the disease in the field. No experiments have been made with other insects, but it is likely that flea beetles, blister beetles, chrysomelids, and many other leaf-eating insects may act as carriers of the disease.

No experiments have been made to determine whether this bacillus can gain entrance to the plant through an uninjured epidermis. Most of the infections probably occur above ground and as the result of insect injuries. Very likely there are some underground infections.

PREVENTIVE MEASURES.

The prompt destruction of all leaf-eating and leaf-puncturing insects is one of the first things to be considered. How this shall be accomplished with the least outlay of time and money is a matter for the economic entomologist to determine.

Of course the more diseased plants in a field at any given time the more possible sources of infection. For this reason diseased plants should be removed and burned with great promptness. This is impracticable after a whole field has become infected, but then the mischief for the season has been accomplished. The time for such precautions is early in the season, when a frequent and thorough search should be made for diseased plants. This can be carried on along with the war of extermination against the insect depredators.

When the disease has become widespread in fields of tomatoes or

eggplants there is no help for it, but in case of potatoes a considerable part of the tubers may be saved if they are dug immediately and stored in a cold dry place. Delay in the harvesting of the tubers for any length of time after the vines have shriveled means simply the infection of all the tubers and the loss of the whole crop, either in the ground or afterwards in the cellar.

This disease sometimes appears on new ground—i. e., ground recently cleared, and occasionally to such an extent as to lead growers to suspect the seed. The seed, however, is not necessarily the carrier of the disease, since beetles with the germs on their jaws can readily fly from one field to another. Such an explanation of the disease does not, however, preclude the possibility of the germ being permanently at home in certain soils or of its being spread by seeds or infected seed potatoes. However this may be, the organism probably lives over winter in the earth of the potato and tomato fields, and therefore such infected soils should be planted to other crops for a series of years before again venturing these two crops or any other solanaceous plants known to be subject to the disease.

To recapitulate, prevention of this disease lies in the direction of the prompt carrying out of the following measures: (1) Early and complete destruction of insect pests; (2) early and complete removal of diseased vines; (3) in case of the potato, the prompt digging of the tubers and their immediate use or storage in a cold dry place; (4) selection of land for subsequent planting which has not been planted in tomatoes, eggplants, or potatoes for several years; (5) selection of tomato and eggplant seeds and potato tubers from plants grown in localities where this disease does not prevail.

MEANS OF DISTINGUISHING BACILLUS SOLANACEARUM FROM KRAMER'S BACILLUS AND THE BACILLUS OF CUCUMBER WILT.

In conclusion it may be of interest to point out (1) some of the differences between the organism here described and that which causes cucumber wilt; and (2) some of the principal differences between this bacillus and the potato-rot bacillus described in 1891, without name, by Dr. Ernst Kramer,[1] and which, for convenience' sake, will here be designated as *Kramer's bacillus*.

[1] Österr. Landw. Centralbl., I, Heft 1, 1891.

Contrast between Bacillus tracheiphilus and B. solanacearum.

Bacillus tracheiphilus.	Bacillus solanacearum.
(1) Extremely viscid in the plant and on various solid media. Strings out in long, delicate threads when touched.	(1) Not sticky in the plant or in culture media, except sometimes slightly so in agar.
(2) Pure white on nutrient agar or potato. Not causing any discoloration of the substratum and indistinguishable from the surface of steamed potato, except by its smooth, wet-shining appearance.	(2) At first dirty white or yellowish white on potato, then brownish, and finally often smoke black. Substratum and fluid in the bottom of the tubes changed to brown. Nutrient agar is browned. The vascular bundles are also browned.
(3) Facultative anaerobic, i. e., with various sugars.	(3) Strictly aerobic so far as yet discovered. Will not grow in closed end of fermentation tube with any of the sugars.
(4) Produces an acid in nutrient media containing grape, fruit, or cane sugar.	(4) Produces no acid with any of the sugars.
(5) A feeble or moderate alkali producer.	(5) An intense alkali producer.
(6) Grows in milk, but induces no visible change, even after many weeks.	(6) Slowly saponifies milk, rendering test tubes of it clear enough to see through.
(7) Odorless in the plant and in culture media.	(7) A decided but not particularly obnoxious odor when grown on potato—the smell of some but not all rotting potatoes.
(8) Will not grow in the thermostat at 37° C.	(8) Grows readily in the thermostat at 37° C.
(9) Thermal death point 43° C. (ten minutes exposure).	(9) Thermal death point approximately 52° C. (ten minutes exposure).
(10) Produces no zoogloœa. Bouillon cultures become cloudy, but not turbid.	(10) Produces zoogloœa in top layers of beef broth or peptone water, in the form of innumerable tiny whitish flecks, which are sometimes slightly inclined to unite into pellicle, but diffuse through the fluid on shaking.

Contrast between Kramer's bacillus and B. solanacearum.

Kramer's bacillus.	Bacillus solanacearum.
(1) Forms in streak cultures on gelatin a leaf-like growth, often difficult to obtain owing to the rapid liquefaction.	(1) Growth on gelatin streak cultures not leaf-like.
(2) Liquefies gelatin rapidly.	(2) Does not liquefy gelatin, or at most (?) does so only very feebly and not until after 5 or 6 weeks.
(3) Produces an abundance of gas (CO_2) in the potato and in nutrient solutions containing grape sugar.	(3) Does not produce gas in the potato or in nutrient solutions with any of the sugars.
(4) Produces butyric acid when inoculated into potato tubers or into glucose solutions containing the necessary nitrogenous substances.	(4) Produces no acid in potato, or in peptone water, or peptonized beef broth to which grape, fruit, or cane sugar has been added. Neutral or slightly acid fluids are speedily rendered alkaline.
(5) Coagulates milk.	(5) Saponifies milk.
(6) Color on various media white to dirty white.	(6) Produces a brown pigment in the host plants, on steamed potato cylinders, and in nutrient agar or peptone water containing grape, fruit, or cane sugar.
(7) At 37° C. in water or moist air speedily converts the whole interior of the inoculated tuber into a soft, wet, white mass, held in a bloated sack consisting of the unruptured skin of the tuber.	(7) When inoculated into the leaves or green stems of the potato plant the organism finds its way into the tubers through the vascular bundles, which are blackened and destroyed. Black or brown cavities are first formed in this part of the tuber, and these slowly or rapidly extend into the starchy parts, but the tuber does not become swollen or give off any gas.
(8) Strong odor of butyric acid and of methylamine and trimethylamine.	(8) A different but not easily described odor—the same as that of some rotting potatoes.

DESCRIPTION OF PLATE I.[1]

Fig. 1. Potato shoot inoculated May 27, 1895. Painted June 11. Bacillus originally from a wilted tomato stem (Southern tomato blight) received from Ocean Springs, Miss. Point of inoculation high on stem at X. The change of color in the diseased stem below the shriveled part is particularly noteworthy.

2. Uninoculated healthy potato shoot from same lot of plants for comparison.

3. An early stage of the rot of the tubers. Plant inoculated June 1, 1896; dug and painted July 20; inoculation by means of a few needle pricks into a vigorous stem 4 feet above the earth; bacillus originally from the stem of a wilted eggplant received from Charleston, S. C.

4. Cross-section of fig. 3. Rot internal, commencing in the vascular bundles; skin of tuber still unbroken. The underground stem bearing this tuber was firm, smooth, and uninjured externally, but it was dark colored, owing to the brown staining of its vascular system, and its vessels were gorged with the bacillus.

5. A very early stage of the rot, a small part of the stem end of the tuber being removed to show its location. Bacilli very numerous, but still confined to the brown tissue in the immediate vicinity of the vascular ring, the latter in open connection with the vessels of the underground stem, which were also gorged with the micro-organisms. Externally there was no indication of disease, and such tubers often find their way to market or into the cellar, the rot becoming visible later on.

6. Longitudinal section of tuber in early stage of rot, showing the location of the decay with reference to the interior of the stem and to the vascular system of the tuber. Plant inoculated June 15, 1896, 3 to 4 feet above ground. Painted August 5.

7. Cross-section of a larger tuber midway between stem end and eye end, showing the restriction of the rot to the vascular system. An early stage of the disease. Tuber from the same plant as fig. 6. There were no external indications of disease in either tuber beyond a slight browning of the underground stems which bore them. Five of the six tubers on this plant were rotted in this manner; the sixth was sound.

8. Cross-section (twice natural size) of stem of potato in same stage of disease as the base of that shown in fig. 1, showing browning of the three principal groups of vessels. Plant inoculated by means of a few needle pricks on the upper part of the stem July 11, 1896. Section painted August 5.

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[1] The plants from which figs. 1 and 3 to 8 of Pl. I were drawn were inoculated in the greenhouse in the presence of numerous control plants, none of which became affected. The tubers here figured were taken from plants which had been dead to the ground for some weeks. In case of the plant which bore the tubers shown in figs. 3 to 5, several other tubers were soft rotten throughout. Another plant in the same pot remained entirely healthy, although the roots of the two plants intertwined and the stems came out of the ground as if all from one plant.

Forbes Co. Boston.

BROWN ROT OF THE POTATO.
(BACILLUS SOLANACEARUM)

D. G. Passmore

DESCRIPTION OF PLATE II.

Fig. 1. Healthy tomato plant from the same lot as fig. 2 (for comparison).

2. Plant inoculated from a pure culture June 15, 1896, by means of needle pricks on upper part of the stem. First symptoms appeared June 19. Photographed June 22. On microscopic examination the vessels 5½ inches below the lowest stabs were found swarming with the bacilli, although externally this part of the stem appeared healthy.

3. Plant inoculated afternoon of July 24, 1896, by placing on it a handful of Colorado beetles (*Doryphora 10 lineata*), which had been fed for some hours on diseased vines derived from pure culture inoculations made July 11. At the time of inoculation the vine was like that shown in fig. 4. The beetles were left on over night and gnawed the plant in many places, but did not injure it seriously. There were no symptoms of disease until after nine days. August 3 to 4 the wilt appeared on fifteen different leaves, resulting from as many different infections. For a day or two the progress of the disease was slow, but after that it was rapid. Photographed August 14. Three weeks after the appearance of the first symptoms the tubers were examined and found to be in various stages of rot.

4. Healthy potato plant from the same lot as fig. 3 (for comparison).

1 2 3 4

BACILLUS SOLANACEARUM n. sp.

1. Healthy tomato plant, for comparison.
2. Tomato plant infected by means of needle pricks.
3. Potato plant infected by means of Colorado potato beetles.
4. Healthy potato plant, for comparison.